J. Anat. (2004) **205**, pp257–266

Tissue-specific expression of the tight junction proteins claudins and occludin in the rat salivary glands

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Abstract

Tight junctions (TJs) are essential features of endothelial barrier membranes and of fluid-secreting epithelial cells, such as in the salivary glands. Novel integral membrane proteins have been identified as components of TJs, namely claudins and occludin. The aim of the present study was to determine the distribution of occludin and claudins in the large salivary glands of the rat. The parotid, submandibular and sublingual salivary glands were harvested from adult Sprague–Dawley rats and cryostat sections were stained using immunoperoxidase and immunofluorescence methods. Claudin-1 was expressed in endothelial cells of microvessels and in short selected segments of the duct system. Claudin-3 was expressed principally in the acinar cells and intercalated ducts, while claudin-4 was principally expressed by the striated and interlobular ducts. Claudin-5 was specific to endothelial cells of microvessels. Occludin was ubiquitously detected in the duct system. Double labelling and confocal microscopy showed some co-localization of claudin-3 with claudin-4, and minimal co-localization of occludin with claudin-4, in the striated ducts. Claudin 2 was not detected in any of the salivary glands. The results indicate specificity of the chemical composition of tight junctions in the rat salivary glands, and may reflect different physiological roles for TJs in the glandular and duct epithelial cells, and in endothelial cells of salivary gland microvessels.

Key words confocal microscopy; immunofluorescence; immunoperoxidase; permeability barriers; transmembrane proteins.

Introduction

The parenchyma of the major salivary glands, parotid, submandibular and sublingual, is divided into lobules, each consisting of a branching system of ducts with peripherally located secretory acini. The saliva elaborated by the acini passes into the lumen of the acini, then through the series of progressively larger ducts. The duct system is well developed in the serous acini-containing glands, such as the parotid and submandibular, and includes three classes of ducts: intercalated, striated and excretory ducts. The principal intralobular ducts are the striated ducts, characterized by vertical 'striations' in the basal portion of the cells. Ultrastuctural studies revealed that the basal striations are folds in the basal membrane, which increase the surface area and house many elongated mitochondria (Ross et al. 2003).

Salivary production occurs in two phases: an acinar phase of production of primary saliva, and a ductal phase of electrolyte reabsorption, resulting in hypotonic saliva (Cook et al. 1998; Guyton & Hall, 2000). Throughout the duct system, active reabsorption of sodium ions occurs at a greater rate than the active secretion of potassium ions. Chloride ions are passively absorbed while bicarbonate ions are actively secreted in exchange for chloride. Thus sodium is depleted from the saliva, establishing a concentration gradient between the luminal fluid in the ducts and tissue fluid. The extensive folding of the basal plasma membrane and the associated mitochondria in the striated ducts epithelial cells are thought to reflect the sodium-pumping capacity of the cell membrane at this location (Dinudom et al. 1993; Cook et al. 1998). Under normal conditions of flow, these ionic changes result in the formation of hypotonic saliva (Guyton & Hall, 2000).

Highly specialized cellular features have evolved to allow cells to form selective barriers and to keep a

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Accepted for publication *9 August 2004*

polarized state between the apical and basolateral membranes of the cell. In epithelial and endothelial cells the tight junctions (TJs) are thought to be the principal structures that contribute to cell polarity, by acting as an intramembrane barrier to prevent lateral movement of membrane proteins that form specific sites in the apical or basolateral membranes. TJs also form the primary barrier against the diffusion of solutes through the paracellular cleft (Anderson, 2001), thus maintaining a selective gradient between the environments in which the apical and basal portions of the same cell are located. In freeze-fracture preparations, TJs appear as aggregates of particles that form continuous anastomosing strands (Staehelin, 1973). The particles are formed of transmembrane proteins (Anderson, 2001). Since the identification of the first TJ protein, ZO-1 (Stevenson et al. 1986) several other TJ-associated proteins have been described (Furuse et al. 1998a; Mitic & Anderson, 1998; Gow et al. 1999; Morita et al. 1999a; Mitic et al. 2000; Anderson, 2001).

Recently, several members of the claudin multigene family of TJ proteins have been described and some of them were shown to confer selective barrier properties on TJs (Mitic & Anderson, 1998; Furuse et al. 1999, 2002; Tsukita & Furuse, 2000; Anderson, 2001). A notable feature of claudins is their ability to induce TJ strands, when singly transfected into fibroblasts (Furuse et al. 1998b; Morita et al. 1999a,b; Tsukita et al. 1999). Claudins show specific patterns of expression in different tissues. Claudin-2, -3 and -5 are expressed in the liver, and each with a selective pattern of distribution (Rahner et al. 2001). Claudin-2 and -4 are expressed in the gut epithelium (Rahner et al. 2001). Claudin-4, -7 and -8 are expressed primarily in the lung and kidney (Morita et al. 1999a) while claudin-2, -3, -4 and -5 are expressed in the pancreas (Rahner et al. 2001). These findings indicate that, in some tissues, there is co-expression of more than one member of the claudin family. In these tissues different claudin species interact with one another and appear to determine specific physiological behaviour of the TJs (Furuse et al. 1999; Tsukita et al. 1999). On the other hand, some types of cells express their own specific claudin species. Claudin-5 was found to contribute to TJs specifically in endothelial cells of blood vessels (Morita et al. 1999b), and claudin-11 is primarily expressed in oligodendrocytes of the brain and Sertoli cells of the testis (Gow et al. 1999), indicating that in these types of cells, TJ strands are mainly composed of a single specific claudin.

Occludin, has a molecular mass of 60–65 kDa, and was also identified as an integral membrane protein morphologically located at TJ strands in epithelial and endothelial cells in various mammalian species (Furuse et al. 1993; Fujimoto, 1995; Saitou et al. 1997). Occludin has four transmembrane domains in its NH_2 terminal half. Its NH_2 and COOH terminals are located in the cytoplasm, and the COOH terminal is bound to the peripheral protein ZO-1 (Furuse et al. 1994). Occludin contributes to the structural (Fujimoto, 1995; Furuse et al. 1996) and functional (McCarthy et al. 1996) properties of TJs. Some studies have shown that occludin is directly involved in the paracellular and the apical–basolateral intramembrane barrier functions of TJs (Balda et al. 1996) and in cell adhesions (Van Itallie & Anderson, 1997). It has been suggested that occludin is involved in the formation of gland-like structures as its expression is reduced in poorly differentiated tumours (Kimura et al. 1997). The aim of the current study is to probe the chemical composition of TJs in the rat salivary glands using antibodies to occludin and claudins.

Materials and methods

Ten male Sprague–Dawley rats (average weight 270 g) were used with ethical approval. Animals were killed under deep anaesthesia induced by intraperitoneal injection of pentobarbital sodium (Nembutal, 60 mg kg^{-1}, CEVA Chemicals Australia Pty Ltd). The thoracic cage was opened, the left ventricle cannulated and the circulation flushed with 200 mL of physiological saline. The parotid, submandibular and sublingual salivary glands were removed, frozen in isopentane cooled in liquid nitrogen and stored at –80 °C. Cryostat sections (10 μm) were cut, collected on silane-coated slides and fixed with cold 50 : 50 acetone/ethanol for 25 min. The sections were immunostained using rabbit anti-claudin-1, -2, -3 and -5 (2 μg mL^{-1}), mouse monoclonal antibodies to claudin-4 and claudin-5 (2 μg mL^{-1}) and rabbit polyclonal antibody to occludin (4 μg mL^{-1}). All antibodies were obtained from Zymed Laboratories Inc., San Francisco, CA, USA.

For the immunoperoxidase method, biotinylated horse anti-mouse and goat anti-rabbit secondary antibodies were used (1 : 200, Vector Laboratories, Burlingame, CA, USA), followed by peroxidase-conjugated streptavidin (1 : 1000, Rockland, Gilbertsville, PA, USA). The peroxidase reaction product was developed with 3,3′-diaminobenzidine tetrahydrochloride (DAB,

0.5 mg mL^{-1}) with 0.03% H_2O_2, for 5 min. For the immunofluorescence method, the secondary antibodies: goat anti-mouse IgG–Alexa Fluor 546 conjugate (1 : 100, Molecular Probes, Eugene, OR, USA) and goat anti-rabbit IgG–FITC conjugate (1 : 100, Sigma Co., St Louis, MO, USA) were used. Sections were examined in an Olympus BX50 microscope fitted with CY3 (red fluorescence), and FITC (green fluorescence) filters. Double labelling with antibodies to claudin-3 and claudin-4, and occludin and claudin-4, was used and confocal images were acquired using a Bio-Rad MRC-1000UV confocal laser scanning microscope, attached to a Nikon Diophot 300 inverted microscope. The excitation wavelength for green was 488/10 nm with emission filter 522/35 nm, and the excitation wavelength for red was 568/10 nm with emission filter 585 LP. The optical slices were 1 μm apart.

Results

Different patterns and distribution were detected for the claudins and occludin in the salivary glands in the present study, but the pattern and distribution for each protein were generally similar in the parotid, submandibular and sublingual salivary glands. Thus the following account applies to all salivary glands unless specified otherwise. Consistent results were found in all ten animals studied and in several staining sessions from the same animal using both immunofluorescence and immunoperoxidase methods.

Labelling for claudin-1 using the immunoperoxidase method showed dark brown reaction product in the blood vessels and certain parts of the duct system in all the salivary glands. In the blood vessels labelling was seen in the capillaries and the intima of arterioles, but was not seen in the venules (Fig. 1A,B). In the duct system moderate labelling was seen in the intercalated ducts and intense labelling was detected in short segments of ducts that link the intercalated and striated ducts (Fig. 1C,D). The acini and striated ducts were not labelled, and the labelling stopped abruptly at the junction of the labelled short segments and the unlabelled striated ducts (Fig. 1C,D). Immunofluorescence showed similar labelling patterns.

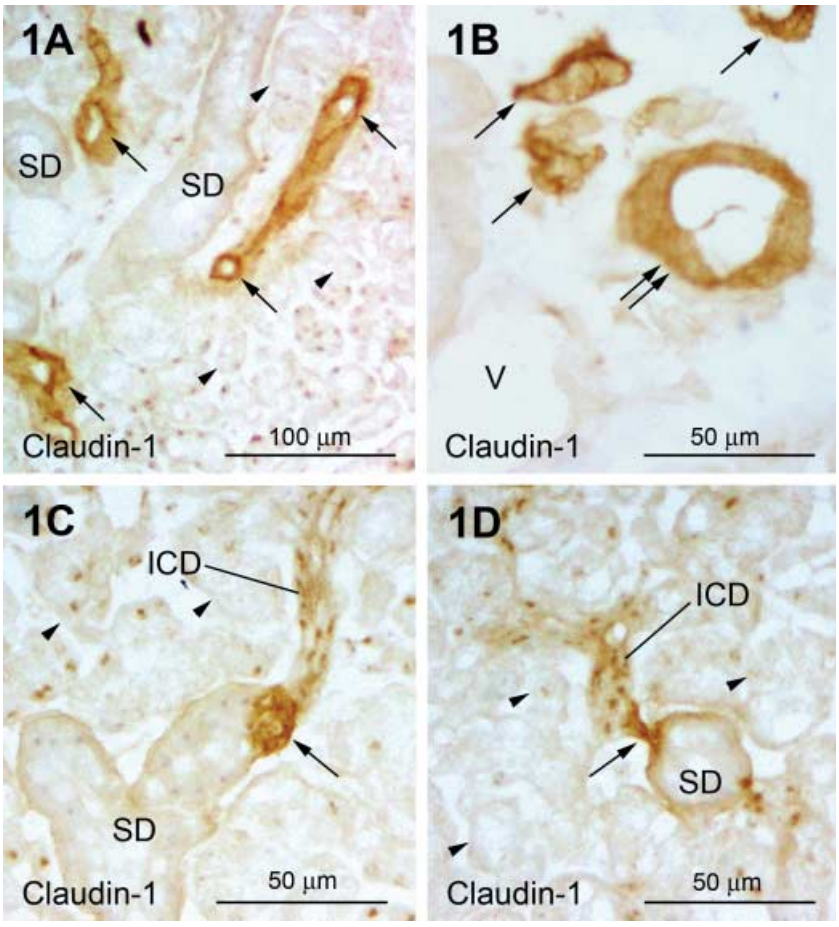

Fig. 1 Light micrographs of cryostat sections from rat parotid salivary glands immunolabelled for claudin-1, using the immunoperoxidase method. Positive labelling for claudin-1 appears as brown reaction product. (A) Strong labelling in endothelial cells of capillaries (arrows) located among the glandular tissue. The acini (arrowheads) and striated ducts (SD) are not labelled. (B) Labelling in interlobular capillaries (arrows) and a larger vessel tentatively identified as arteriole (double arrows). The capillaries and arteriole are cut obliquely and show the stained endothelial cell layer. However, an associated larger vessel, tentatively identified as a venule (V) is not labelled. (C,D) Moderate labelling in intercalated ducts (ICD) and strong labelling in short segments (arrows) that link intercalated and striated ducts (SD). The acini (arrowheads) and striated ducts (SD) are not labelled.

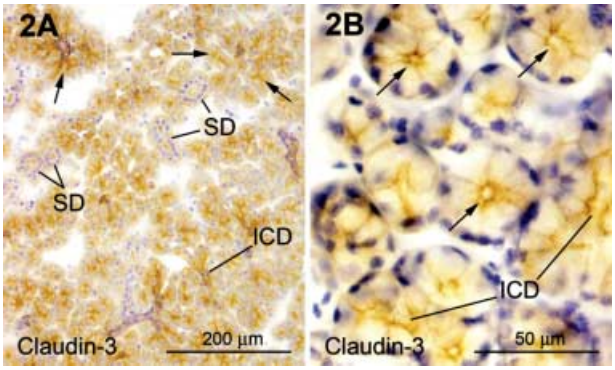

Fig. 2 Light micrographs of cryostat sections of rat salivary glands immunoperoxidase labelled for claudin-3. Positive labelling appears as dark brown reaction product. The nuclei are stained blue with haematoxylin. (A) Low-magnification micrograph of parotid gland showing labelling for claudin-3 in the central regions of acini (arrows) and in intercalated ducts (ICD). Epithelial cells of the striated ducts (SD) are faintly labelled in the central region near the lumen. (B) High-magnification micrograph of sublingual gland showing strong labelling for claudin-3 in the apical and lateral membranes of acinar cells (arrows) and intercalated ducts (ICD).

Immunostaining for claudin-3 using the immunoperoxidase method showed dark brown peroxidase reaction product indicating positive labelling in the parotid, submandibular and sublingual salivary glands (Fig. 2). Claudin-3 was principally located in the small intercalated ducts and the lateral membranes of acinar epithelial cells, especially near their luminal end (Fig. 2A,B). Less intense labelling was seen in epithelial cells of the striated (Fig. 2A) and excretory ducts. Blood vessels were not labelled for claudin-3. Immunofluorescence and confocal microscopy confirmed these findings, where fluorescence was principally seen in the lateral membranes of acinar epithelial cells and was faintly present in striated duct epithelial cells (Fig. 3B,D).

Immunostaining for claudin-4 using the immunoperoxidase method showed selective distribution of claudin-4 in the striated and large excretory ducts, but not in the acini or intercalated ducts (Fig. 3A). Labelling was principally seen in the lateral and basal membranes of duct epithelial cells (Fig. 3A). No labelling of any other cells in the parenchyma or the blood vessels was seen. Labelling of the basal striations of duct epithelial cells for claudin-4 was clearly seen using the immunofluorescence method (Figs 3C and 4B). Double immunofluorescence labelling for claudin-3 and claudin-4 and confocal microscopy showed the predominant distribution of claudin-3 in the acini (Fig. 3B), and the selective distribution of claudin-4 in the striated ducts (Fig. 3C), with some co-localization of the two proteins in the striated duct epithelial cells (Fig. 3D).

Labelling of the salivary glands for claudin-5 using rabbit polyclonal or mouse monoclonal antibodies showed similar results. Claudin-5 was detected only in capillaries (Fig. 4A) and in the endothelial cell layer of arterioles (Fig. 4C), but venules and veins were not labelled. Acinar and duct epithelial cells did not show any labelling for claudin-5. Double labelling for claudin-5 and claudin-4 showed no co-localization of the two proteins (Fig. 4A,B).

Immunostaining using the peroxidase method detected occludin in all the salivary glands. Faint labelling was seen in the central portion of acinar epithelial cells (Fig. 5A). By contrast, intense labelling was seen in the intercalated, striated and large excretory ducts (Fig. 5A,B). In the striated ducts labelling was particularly evident in the lateral membranes with higher staining intensity near the lumen (Fig. 5A,B). Immunofluorescence confirmed the localization of occludin as detected by the peroxidase method. Double immunofluorescence labelling for occludin and claudin-4 showed minimal co-localization in the striated ducts, occludin being mainly seen near the luminal end of lateral membranes and claudin-4 predominantly located in the basal membranes (Fig. 5C–E). Positive labelling for occludin was also seen in the arterioles located in the connective tissue sheath and septa within the gland, where the labelling appeared in the smooth muscular layer, but labelling was not seen in endothelial cells of these vessels (Fig. 5F).

Antibody to claudin-2 did not show any labelling in the parotid, submandibular or sublingual salivary glands. In the labelling procedure for all other proteins, omission of the primary antibody in the negative control sections eliminated all labelling (Fig. 4D).

Discussion

Molecular explanations for the variable physiological properties of TJs in different tissues have started to unfold. Recent studies have documented selective distribution of individual claudins in TJs in various tissues, and circumstantial evidence suggests that claudins play a major role in the variability of barrier functions of TJs (Mitic et al. 2000; Anderson, 2001; Rahner et al. 2001). In the present study we asked whether the claudin family members are present in the salivary glands of the

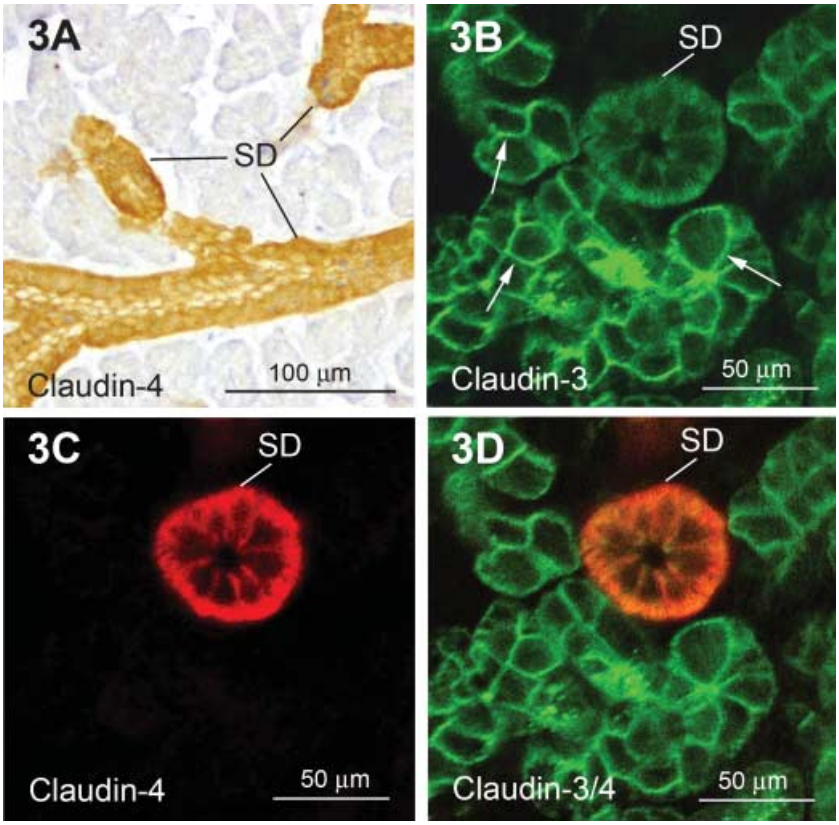

Fig. 3 (A) Light micrograph of a cryostat section from rat parotid gland labelled for claudin-4 using the immunoperoxidase method. Dark brown reaction product indicating positive labelling is seen in the striated ducts (SD) but not in the surrounding acini. In the striated ducts, the labelling is seen principally in the cell membranes, thus outlining the boundaries of epithelial cells. (B–D) Confocal images of a cryostat section of rat parotid gland double labelled for claudin-3 using rabbit polyclonal antibody and claudin-4 using mouse monoclonal antibody. Labelling of claudin-3 (B) appears as green fluorescence, through an FITC filter, in the lateral membranes of acinar epithelial cells (arrows) and to a lesser extent in epithelial cells of a striated duct (SD). Labelling for claudin-4 (C) appears as red fluorescence in epithelial cells of the striated duct (SD) at their lateral membranes and at the basal striations. (D) Merged confocal images of claudin-3 (B) and claudin-4 (C) and displays faint yellow coloration especially in the basal part of the striated duct (SD) indicating some co-localization of the two proteins.

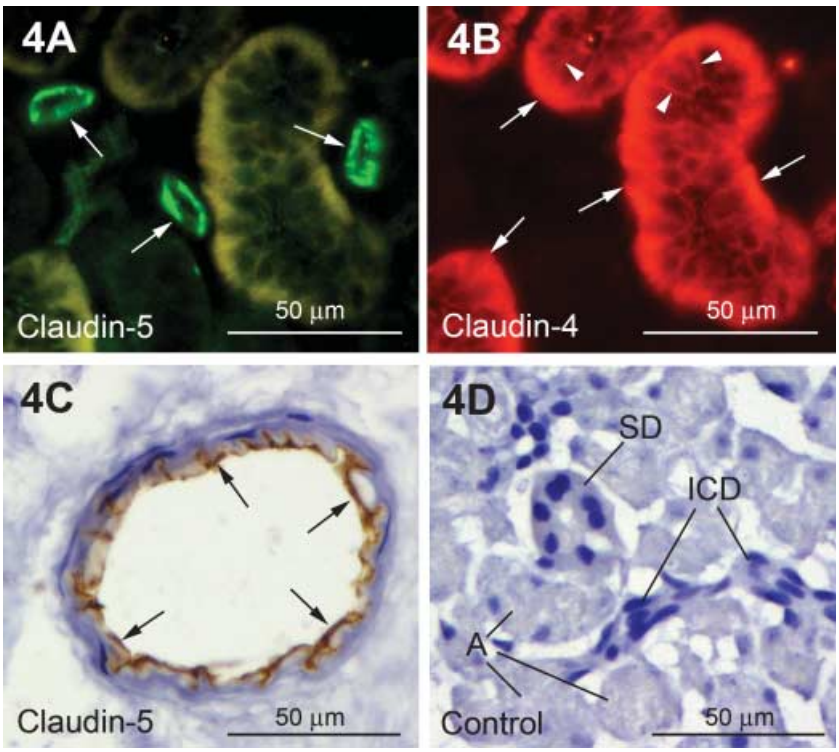

Fig. 4 (A,B) Light micrographs of a cryostat section from rat submandibular gland showing double immunofluorescence labelling, using rabbit polyclonal antibody for claudin-5 and mouse monoclonal antibody for claudin-4. Using an FITC filter (A), labelling for claudin-5 appears as green fluorescence in capillaries (arrows). Using a CY3 filter (B) labelling for claudin-4 appears as red fluorescence in a group of striated ducts. The labelling for claudin-4 appears at the lateral membranes (arrowheads) and is particularly intense at the basal striations (arrows) of duct epithelial cells. (C) Light micrograph of parotid gland immunostained for claudin-5 using the peroxidase method. Brown peroxidase reaction-product, indicating positive labelling for claudin-5, is seen in endothelial cells (arrows) of an interlobular arteriole. The section is counterstained with haematoxylin. (D) Negative control section of parotid gland immunostained without any primary antibody. No brown peroxidase reaction product is seen. The section was counterstained with haematoxylin and shows intercalated ducts (ICD), a striated duct (SD) and acini (A).

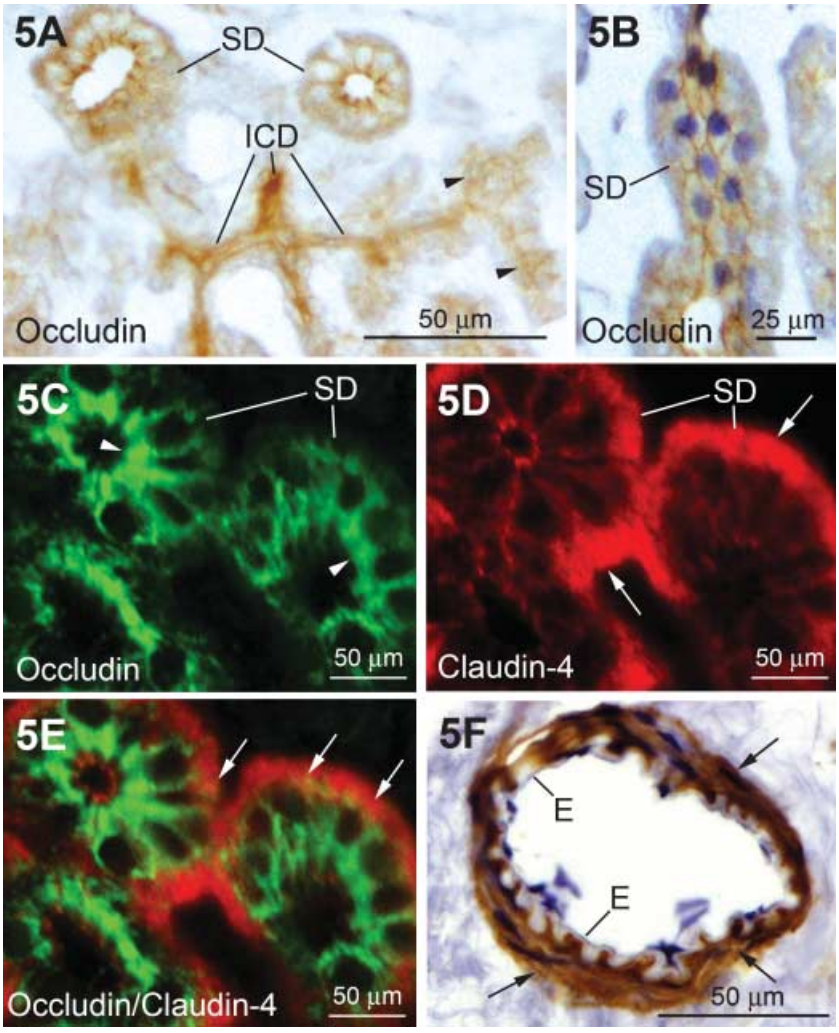

Fig. 5 (A,B,F) Cryostat sections of rat parotid glands immunostained for occludin using the immunoperoxidase method. Dark brown peroxidase reaction product indicates the presence of occludin. (A) Faint labelling in the central part of acinar cells (arrowheads). Intense labelling is seen in intercalated ducts (ICD) and in the lateral membranes of striated duct (SD) epithelial cells, especially near their luminal end. (B) Labelling of the lateral membranes of epithelial cells in a tangential section of a striated duct (SD) giving a mosaic appearance. (C–E) Confocal images of a cryostat section of submandibular gland immunolabelled for occludin and claudin-4 using the immunofluorescence method. Occludin labelling appears as green fluorescence (C) and claudin-4 appears as red fluorescence (D). Occludin labelling is seen in the lateral membranes of striated duct (SD) epithelial cells especially near their luminal end (arrowheads) and to a lesser extent in the basal membranes. Claudin-4 labelling appears principally in the basal membranes (arrows) of striated duct (SD) epithelial cells. (E) Merged images in C and D with minimal co-localization of occludin and claudin-4 in the basal part of duct epithelial cells as indicated by a faint yellow coloration (arrows). (F) Positive labelling for occludin in a small interlobular artery. Labelling appears in the tunica media (arrows) located external to the corrugated internal elastic lamina. The endothelial (E) cell layer is not labelled.

rat, and compared their distribution with another TJ protein, occludin. We found that, claudin-1, -3, -4 and -5 and occludin are expressed in all the major salivary glands of the adult rat but with selective distribution patterns.

In the salivary glands, the acini and their related consecutive duct segments, the intercalated, striated and large interlobular ducts, act in concert as functional units, with each segment having a specific contribution to the formation of the final salivary product. The modification of saliva must be regulated by tissue-specific TJs, with permissive and barrier mechanisms, to achieve chemical specificity of the saliva. In the present study certain claudins and occludin were expressed in various segments along the production route. In the acini and intercalated ducts claudin-3 was the principal claudin. Short truncated segments of the intercalated ducts that join the striated ducts showed specific expression of claudin-1, while the striated and interlobular ducts showed mainly expression of claudin-4. Occludin showed ubiquitous expression in epithelial cells, with progressive increase in the level of expression from the acini to the striated ducts. The peripheral to central expression of these TJ proteins in the salivary units may form the basis for the progressive tightening of the epithelium, and may be similar to a proximal to distal tightening seen in other tubular epithelia such as the intestine (Rahner et al. 2001) and the nephron (Kiuchi-Saishin et al. 2002).

Evidence exists for the functional significance of some members of the claudin family. Paracellin-1 (claudin-16) is required for the physiological renal resorption of Mg^{2+} (Simon et al. 1999). In claudin-11/OSP-deficient mice TJ strands are absent in the myelin sheath of oligodendrocytes and in Sertoli cells, and the mice show demyelination and are infertile (Gow et al.

1999). Claudin-1 is a component of epithelial and endothelial TJs (Furuse et al. 1998a). Although the exact physiological role of claudin-1 is not clear, strong evidence points to its importance in the normal function of tissues. Cl-1–/– deficient mice show an alteration in the skin, where newborn mice develop severe dehydration and die within 1 day of birth (Furuse et al. 2002). Conversely, over-expression of claudin-1 in Madin–Darby canine kidney (MDCK) cell monolayer increases their transepithelial electrical resistance (McCarthy et al. 2000). In the present study claudin-1 was detected in endothelial cells of salivary gland microvessels and in selected duct segments that link the intercalated and striated ducts. The significance of the selective distribution of claudin-1 in restricted parts of the duct system, and its strong expression in capillaries and endothelial cells of arterioles but not veins, is yet to be clarified by functional studies.

In the present study claudin-4 was detected in the striated and the large excretory ducts in the three major salivary glands of the rat. Previous studies on cell lines suggested that claudin-4 forms pores through the TJ that discriminate against sodium ions but are indifferent to chloride ions (Van Itallie et al. 2001, 2003). Over-expression of claudin-4 in cultured MDCK II cells resulted in decreased paracellular electrical conductance owing to a selective decrease in Na^+ permeability (Van Itallie et al. 2001, 2003). In the salivary glands sodium absorption from the primary saliva occurs in the duct system, in the process of formation of hypotonic saliva, particularly in the basal portion of the striated duct cells (Dinudom et al. 1993). The present study demonstrates the first morphological evidence for the localization of claudin-4 in the rat salivary glands and provides a molecular explanation for the high sodium pumping ability of the striated ducts, suggested as a mechanism of production of hypotonic saliva.

MDCK II cells that normally express claudin-2 have low transepithelial electrical resistance (TER), a measure of the tightness of TJs (Furuse et al. 2001). Induced expression of claudin-2 in MDCK I cells, which normally lack claudin-2 and have high TER, results in a dramatic drop in electrical resistance (Furuse et al. 2001). Analysis of TJ proteins was carried out in two clones of MDCK cells, C7 that have high TER, and C11 that show low TER. Claudin-2 was found to be only marginally expressed in C7 cells, but markedly expressed in C11 cells (Amasheh et al. 2002). In addition, induced expression of claudin-2 in C7 cells decreased their TER, with selective increased paracellular conductivity for sodium and potassium ions, but not chloride ions, dextran, mannitol and lactulose, suggesting that claudin-2 forms cation-selective channels in TJs of these cells (Amasheh et al. 2002). In the present study claudin-2 was not detected in any of the salivary glands. Thus its absence from striated duct epithelial cells and the presence of claudin-4, which restricts the passage of sodium, indicate a cation-tight paracellular pathway in the duct system of the salivary gland. This is consistent with the suggested role of striated ducts in the physiological production of hypotonic saliva.

Claudin-3 is a constituent protein of TJs in epithelial and endothelial cells. In epithelial TJs, claudin-3 was found in equal amounts in culture cells that showed different levels of transepithelial resistance (Amasheh et al. 2002). In addition, transfection experiments showed no relationship between electrical transepithelial resistance and claudin-3 (Furuse et al. 2001). From the above studies it seems that claudin-3 is not involved directly in barrier properties of epithelial cells. By contrast, claudin-3 was selectively lost in endothelial cells of the compromised blood–brain barrier in an *in vivo* mouse model of experimental allergic encephalomyelitis, and in leaky vessels of human brain tumours (Wolburg et al. 2003). The present study showed that claudin-3 in the major salivary glands of the rat was expressed in the luminal and lateral membranes of epithelial cells of the acini, and the intercalated and striated ducts. It is likely that in the salivary glands, claudin-3 forms a large fraction of the TJ proteins, because of its wide distribution in the acini and ducts, although at variable concentrations in these regions. In the present study claudin-3 was not detected in the blood vessels of the salivary glands. The specific function of claudin-3 and the significance of its differential distribution in epithelial cells of the acini and ducts, and its absence from endothelial cells in the salivary glands are yet to be elucidated.

Endothelial cells have important functions in determining and regulating vascular permeability, because they provide the interface between the blood and tissue microenvironment. In the salivary glands, water and solutes destined to become exocrine secretion must pass across three different barriers: the vascular endothelium, the glandular interstitium and the secretory epithelium (Smaje & Henderson, 1984). Thus endothelial cells of microvessels of the salivary glands

contribute to a selective barrier between the blood and extracellular fluid, in which the acini and ducts are bathed. Claudin-5 is present at high specificity in endothelial cells and is possibly involved in the function of endothelial cell TJs, although different patterns of expression are seen among endothelial cells of vessels in different organs (Morita et al. 1999b, 2003). In the present study, the detection of claudin-5 in endothelial cells of all the major salivary glands, but not in parenchymal cells, supports the specificity of claudin-5 to endothelial cells (Morita et al. 1999b). Interestingly, in the present study, not all endothelial cells in the salivary vessels were labelled for claudin-5; unlike those of capillaries and arterioles, endothelial cells of veins and venules were not labelled. This finding is consistent with the results of Morita et al. (1999b), who found that claudin-5 is an endothelial cell-specific component of the TJs in arterioles and arteries but is not expressed in endothelial cells of veins.

The role of occludin in the structure and function of TJs requires further investigation owing to apparently contradicting reports in the literature. It has been reported that occludin-deficient embryonic stem cells can differentiate into polarized epithelial cells with TJs that are functionally able to exclude low-molecular-mass tracers (Saitou et al. 1998). Occludin appears not to be the main component of TJ strands, as its transfection into fibroblasts leads to the formation of only short strands of TJs (Furuse et al. 1998b; Saitou et al. 1998). Furthermore, occludin was not detected in TJs of dermal vessels, which are predominantly formed of claudin-5 (Morita et al. 2003). Occludin is also absent in TJs of the lung and kidney (Morita et al. 1999b). By contrast, evidence appears to be in favour of occludin making an important contribution to the structure and function of TJs. It has been suggested that occludin is involved in the regulation of paracellular permeability (Balda et al. 1996, 2000). Expression of occludin in MDCK strain II cell line results in increased TER. Paradoxically, despite the increase in TER these cells show an increase in paracellular flux of small molecular dextran tracer (Balda et al. 1996), suggesting a role for occludin in the formation of selective pores despite the high electrical resistance. It has also been suggested that occludin may co-operate with other transmembrane components to allow selective paracellular diffusion. An association between occludin and claudin-4 is suspected to regulate selective paracellular permeability (Balda et al. 2000). In the present study occludin was detected in the lateral membranes of striated duct epithelial cells, and confocal microscopy showed intense distribution in the lateral membranes near their luminal end, a characteristic location of epithelial TJs. Furthermore, the present study showed that striated duct epithelial cells express occludin and claudin-4. However, the two proteins were expressed predominantly in different domains; occludin mainly in the luminal portion of membranes with a reducing gradient towards the basal membranes, while claudin-4 had an opposite pattern of distribution with minimal co-localization of the two proteins.

The detection of occludin in the luminal membranes of duct epithelial cells in the present study was unexpected, because a previous study using freeze fracture immunocytochemistry (Fujimoto, 1995) showed its association with tight junction strands, which are principally located in the lateral membranes of the cells. However, its additional localization to luminal membranes of duct epithelial cells seen in the current study suggests an initial site of insertion in the membranes prior to mobilization to the lateral membranes for assembly in TJs. Alternatively, occludin may be involved in other cell membrane functions. This is of particular interest as a recent study of phenotypes of mice lacking occludin showed loss of cytoplasmic granules in striated duct epithelial cells in the salivary glands (Saitou et al. 2000).

Occludin has also been implicated in the regulation of tight junctional permeability in endothelial cells (Hirase et al. 1997). Occludin is strongly expressed in adult guinea-pig brain endothelial cells, morphologically appears as a continuous line at cell–cell contacts, and its level of expression increases in rat brain development, from postnatal day 8 to postnatal day 70 (Hirase et al. 1997). However, in the present study of the salivary glands, occludin was not detected in endothelial cells. This may reflect heterogeneity in the expression of this protein in vessels of the brain and salivary glands and may be related to different functional roles for TJs in these two vascular beds. This interpretation may be supported by the weak expression of occludin in vessels of tissues that lack an endothelial barrier such as the tongue (Hirase et al. 1997) and the dermis (Morita et al. 2003). An additional finding in the present study was the detection of occludin in the muscular layer of salivary gland arterioles but the functional significance of this selective distribution is not known.

Acknowledgements

We thank Professor Brian Setchell and Gail Hermanis for critical reading of the manuscript, and Meredith Wallwork for assistance with confocal microscopy. Dr Marcello Peppi was supported by an Italian FIRB Grant number RBAUO1CCAJ-005.

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